

**CERTIFICATE OF MAILING**

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231.

Typed or Printed Name	Donna Macedo		
Signature	<i>D. Macedo</i>	Date	5/29/01

<b>DECLARATION UNDER 37 C.F.R. § 1.132</b>  Address to: Assistant Commissioner for Patents Washington, D.C. 20231	Attorney Docket Confirmation No.	CLON-015
	First Named Inventor	Chenchik et al.
	Application Number	09/440,829
	Filing Date	November 15, 1999
	Group Art Unit	1655
	Examiner Name	Forman, B.
	Title	Long Oligonucleotide Arrays

Sir:

I, Alex Chenchik, am a co-inventor of the above referenced application and an employee of Clontech Laboratories, Inc., the assignee of the above the above referenced application. A copy of my C.V. is enclosed.

I hereby declare the following:

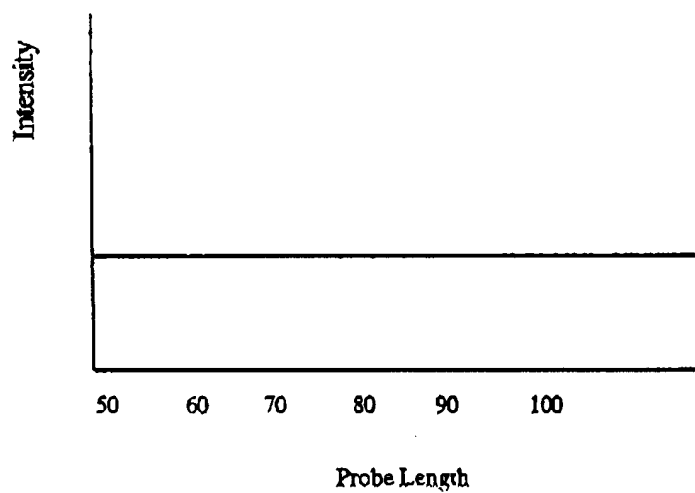
In the working exemplification of the above captioned application, an array is prepared in which the probe lengths range from 50 to 100 nt. See Examples 1 to 5 of the above captioned application.

In Example 6, the hybridization efficiency of each of the different probe lengths on the array was assayed and the results are graphically provided in Fig. 1 of the application.

Prior to conducting the assay of Example 6, it was my expectation, which is the same as with what those of skill in the art would expect, that the hybridization efficiency would be the same regardless of probe length. As such, I expected to obtain results that would graph as follows:

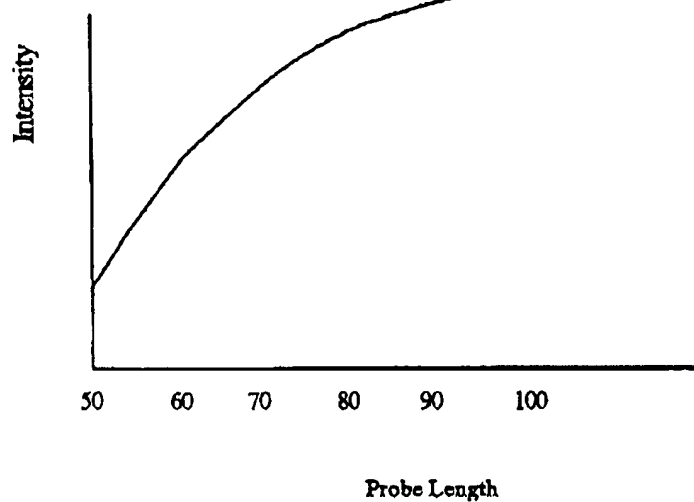
Atty Dkt. No.: CLON-015  
USSN: 09/440,829

Expected graph:



Instead of observing the above expected results, the following unexpected results were obtained:

Observed graph:



As such, using a probe length of 50 to 100 nt provides unexpected results.

Atty Dkt. No.: CLON-015  
USSN: 09/440,829

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 5/25/01

Signature: 

Alex Chenchik

enc

C Figure 1

C C.V. of Alex Chenchik

Atty Dkt. No.: CLON-015  
USSN: 09/440,829

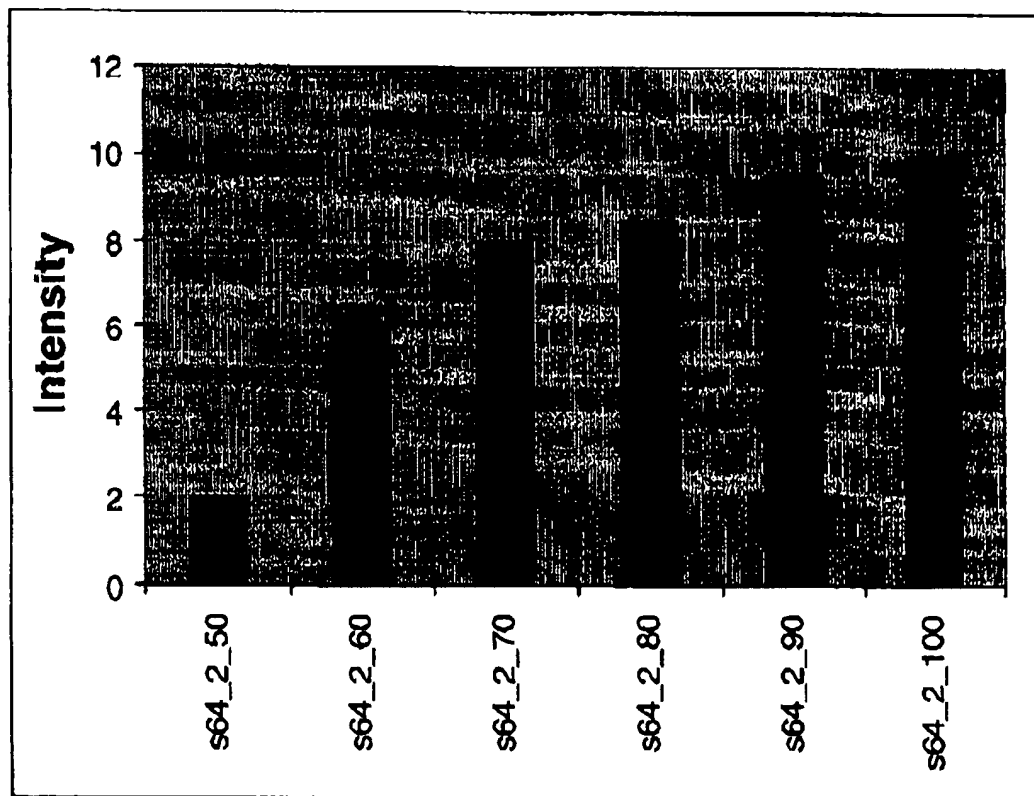
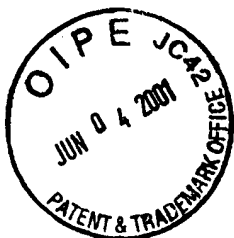


FIGURE 1



## CURRICULUM VITAE

ALEXANDR A. TCHENTCHIK, Ph.D.

### EDUCATION

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Ph.D. degree in Molecular Biology, Institute of Molecular Biology, USSR Academy of Sciences, Moscow, 1982.

Thesis: " Molecular topography of RNA polymerase-lacUV5 promoter complex".

M.S. degree in chemistry. Institute of Fine Chemical Technology, Moscow, 1978.

Thesis: " Chemical modification of E.coli RNA polymerase".

### EXPERIENCE

-----  
1998 – 1999      Director of Array Program, Gene Cloning and Analysis Department,  
CLONTECH Laboratories, Inc. (1020 East Meadow Circle, Palo  
Alto, CA94303.

1997 – 1998      Associate Director, Gene Cloning and Analysis Department,  
CLONTECH Laboratories, Inc. (1020 East Meadow Circle, Palo  
Alto, CA94303.  
Development of new technologies for cDNA/oligonucleotide-based  
expression arrays, RNA chip, SMART cDNA amplification, RecA-  
based full-length cDNA cloning.

1994 - 1996      Research Scientist, PCR Group ( Cloning II ),  
CLONTECH Laboratories, Inc. ( 4030 Fabian Way, Palo Alto,  
CA94303.  
Development of new technologies for cDNA library  
construction, cDNA cloning, genome walking & mapping, cDNA &  
genomic subtraction, finding differentially expressed genes ( RNA  
fingerprinting, differential display ), long-distance PCR, RT-PCR,  
aptamer selection, RNA isolation.

1992 - 1994      Director of Research Dept.,  
"Technogene" company, National Cardiology Research  
Center, Moscow, Russia; International Distributor of  
"CLONTECH Laboratories, Inc.", 4030 Fabian Way, Palo  
Alto, CA 94303, USA.  
Leader of joint research projects with Clontech:  
Large-scale RNA/DNA purification, New PCR technologies,  
cDNA library construction, development of new cloning  
vectors, chemical reagents for oligonucleotide synthesis,

MTW blots. Development of new methods for large-scale RNA isolation, analysis of poly(A)RNA composition, searching of differentially expressed genes, cDNA library construction. Investigation of new nucleotide analogues for reverse transcriptase, Tag DNA polymerase, DNA polymerase I.

- 1984-1992 Senior Scientist,  
Genetic Engineering Lab., National Cardiology Research Center, Moscow, Russia.  
Regulation of gene expression at the course of F9 cell differentiation, in situ hybridization, cell-free system of RNA polymerase II transcription, crystallization of E.coli RNA polymerase.
- 1983-1984 Junior Scientist,  
Genetic Engineering Lab., National Cardiology Research Center, Moscow, Russia.  
Cloning of plasminogen activator ( urokinase) gene,  
Regulation of gene expression during F9 cell differentiation
- 1978-1983 Postgraduate Student,  
Institute of Molecular Biology, USSR Academy of Sciences,  
Moscow,  
Russia.  
Investigation of topography of RNA polymerase, lac repressor - lacUV5 promoter complexes by chemical cross-linking approach, Mechanism of RNA polymerase elongation, Development of methods for large scale purification of RNA polymerase E.coli, DNA polymerase I E.coli, T4 polynucleotide kinase, T4 DNA ligase, T4 RNA ligase
- 1974-1978 Undergraduate and M.S. trainee,  
Institute of Molecular Biology, USSR Academy of Sciences,  
Moscow,  
Russia. Chemical modification of E.coli RNA-polymerase.

## METHODS

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Gene cloning, DNA sequencing, PCR, cDNA library construction/screening, hybridization of nucleic acids, DNA/RNA probes, plasmids/phages microbiology. Chromatography/HPLC/Electrophoresis and others methods of purification/analysis of DNAs/RNAs/Proteins. Cell culture including gene transfection technology.

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3. Rozovskaya T.A., Chenchik A.A., Bibilashvilli R.Sh. Pyrophosphorolysis reaction catalyzed by *Escherichia coli* RNA-polymerase. *Mol.Biol.(Russia)*, 1981, v.15, 636-652.
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9. Gragerov A.A., Chenchik A.A., Aivazashvilli V.A., Bibilashvilli R.Sh., Nikiforov V.G. *Mol. Gen. Genet.*, 1984, v.21, 305-317.
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11. Chenchik A.A., Diachenko L.B., Bibilashvilli. Quantitative analysis of individual RNAs in mammalian poly(A)RNAs. *Mol. Biol.(Moscow)*, 1992, v.26, 784-793.
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13. Chenchik A.A., Diachenko L.B., Bibilashvilli R.Sh. Application of poly(A)RNA patterns method for searching of differentially expressed genes. *FEBS Letters*, 1993, v.324, 136-139.
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phosphodiesterase. I. Mol. Biol., 1993, v.27, 67-74.

16. Kellogg D.E., Rybalkin I., Chen S., Mukhamedova N., Vlasik T., Siebert P.D., Chenchik A. TaqStart antibody: "Hot Start" PCR facilitated by a neutralizing monoclonal antibody directed against Taq DNA polymerase. *BioTechniques*, 1994, v.16, 1134-1137.
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18. Paul D. Siebert, Alex Chenchik, David E. Kellogg, Konstantin A. Lukyanov and Sergey A. Lukianov. An improved PCR method for walking in uncloned genomic DNA. *Nucl. Acids Res.*, 1995, v.23, 1087-1088.
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21. Diatchenko, L., Lau, Y-F, C., Campbell, A., Chenchik, A., Moqadam, F., Huang, B., Lukyanov, S., Lukyanov, K., Gurskaya, N., Sverdlov, E., and Siebert, P.D. (1996) Suppression Subtractive Hybridization: A method for generation of differentially regulated or tissue-specific cDNA probes and libraries. *Proc. Natl. Acad. Sci. USA* 93:6025-6030.
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23. Lukyanov, K., Gurskaya, N., Matz, M., Khaspekov, G., Diatchenko, L., Chenchik, A., Iljevich-Stuchkov, S.G. and Lukyanov, S. (1996) Method for obtaining normalized cDNA libraries based on PCR suppression effect. *Bioorganic Chemistry, (Russian)*, 22:686-690.
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26. Zhang, W., Chenchik, A., Chen, S., Siebert, P. and Rhee, C.H. (1997) Molecular Profiling of human gliomas by cDNA expression array. *J. of Genet. Medicine.*, 1:57-59.
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28. Diatchenko, L., Chenchik, A. and Siebert, P. Suppression Subtractive Hybridization: A method for Generating Subtracted cDNA Libraries Starting from Poly(A+) or Total RNA. In RT-PCR Methods for Gene Cloning and Analysis. (1999) Ed. by P. Siebert and J. Larrick, BioTechniques Books, Natick, MA., p.213-238.
29. Chen, S.S., Chenchik, A., Lukianov, K.A. and Siebert, P. Improved Technique for walking in Uncloned Genomic DNA. In RT-PCR Methods for Gene Cloning and Analysis. (1999) Ed. by P. Siebert and J. Larrick, BioTechniques Books, Natick, MA., p.289-302.



## BIOGRAPHICAL SKETCH

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Palo Alto, CA 94303-4230, U.S.A.

## SCIENTIFIC BACKGROUND

Dr. Alex Chenchik obtained his Ph.D. degree in Molecular Biology (1982) in Professor Robert Bibilashvili's laboratory at the Institute of Molecular Biology (Moscow, Russia) where he worked for three years as a post graduate student. His thesis, "Molecular topography of RNA polymerase-lacUV5 complex", examined the three-dimensional structure of complexes of the *Escherichia coli* RNA polymerase with lac promoter.

After receiving his Ph.D., Dr. Chenchik together with Professor Bibilashvili set up a new laboratory of Genetic Engineering in National Cardiology Research Center (Moscow, Russia) in 1984. As a research scientist and later as a senior research scientist he has been involved in cloning and generation of *E. coli* overproducer strain of human urokinase for pharmaceutical industry, regulation of gene expression in the course of embryonal carcinoma cell differentiation, searching and investigation of new nucleotide analogues for reverse transcriptase, Taq DNA polymerase and DNA polymerase I. He developed a several novel methods for large-scale RNA isolation, searching of differentially expressed genes based on reverse transcriptase extension assay, cDNA library construction, cDNA subtraction and "hot-start" PCR based on using monoclonal antibodies against Taq DNA polymerase.

In April 1994, Dr. Chenchik joined PCR group at CLONTECH as the Research Scientist in charge of the development of PCR-based products for gene cloning and analysis. In January 1995 he introduced the Marathon™ cDNA Amplification kit and latter Marathon-Ready cDNAs for fast PCR-based cloning of full-length cDNAs. Dr. Chenchik has been participate in the development of the TaqStart™ antibodies technology for "hot-start" PCR, Advantage™ PCR Amplification Kits for long-distance PCR, PCR-Select™ cDNA Subtraction Kit for selective amplification of differentially expressed genes, Promoter Finder™ for PCR-based cloning of promoter regions, CapFinder™ PCR cDNA Library Construction Kit for generating high-quality cDNA libraries from nanogram amount of total RNA and Atlas™ cDNA Expression Array for high throughput gene expression analysis. Dr. Chenchik is author of 27 scientific publications and 5 patents. Currently he is the leader of the group of 30 people responsible for development of novel technologies for cDNA cloning and expression analysis at CLONTECH.

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1. Chenchik A.A., Bibilashvilli R.Sh. Chemical modification of RNA polymerase by dimethylsulfate. *Mol. Biol. (Russia)*, 1977, v.11, 403-409.
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  28. Diatchenko, L., Chenchik, A. and Siebert, P. *Suppression Subtractive Hybridization: A method for Generating Subtracted cDNA Libraries Starting from Poly(A+) or Total*

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